Example 10

Suppression of Caffeine Synthesis According to an Antisense Method

[0097] A recombinant vector carrying an antisense N-methyl transferase gene was constructed by the following procedure:

[0098] DNA fragments were amplified by PCR using the total length of the isolated N-methyl transferase gene as used in Example 9 as the template and the primer having the nucleotide sequences of ID SEQ NOs: 20 and 21, respectively. The ends of the DNA fragments thus amplified were changed into the blunt ends by BKL kit (TAKARA) to obtained the blunt-ended PCR-amplified fragments.

[0099] Separately, pBI vector (Clontech), to which a hygromycin resistance gene was connected, was cut with XbaI and SacI to remove the β-glucuronidase gene and the ends of the linear vector thus obtained were changed into the blunt ends.

[0100] The blunt-ended linear vector was ligated with the blunt-ended PCR amplified fragments to obtain recombinant vectors by Ligation Kit (TAKARA), and then the vector carrying the desired N-methyl transferase gene which was inserted in the reverse direction at the operable location on the downstream side of the CaMV35S promoter in the pBI vector was selected from the reaction products by sequencing. Thus, the desired recombinant vector, into which the antisense N-methyl transferase gene was inserted, was obtained and used the following transformation.

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